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Nutritional Contents and Antioxidant Properties of *Pleurotus florida* (Fr.) Kumm. and *Hypsizygus ulmarius* (Bull. ex Fr.) Redhead

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ABSTRACT

The two edible mushrooms, *Pleurotus florida* and *Hypsizygus ulmarius*, were assessed for nutritional value in proximate composition such as ash, moisture, carbohydrates, protein, fat, fiber and vitamins C (L-ascorbic acid), B1 (thiamine), B2 (riboflavin), vitamin B9 (folic acid), vitamin B5 (Pantothenic acid) and vitamin B3 (niacin), minerals, zinc (Zn), copper (Cu), potassium (K), sodium (Na), iron (Fe), calcium (Ca), magnesium (Mg) and Phosphorus (P). To assess antioxidant potential, different assays namely reducing power assay, hydrogen peroxide scavenging (H2O2) assay, and 2,2-diphenyl-1- picrylhydrazyl (DPPH) assay with different concentration of 100, 200, 300, 400 and 500 µg/ml of extracts were performed. The antioxidant activities were determined with standard, ascorbic acid and IC50 values of aqueous and methanol extract showed a strong antioxidant capacity. The maximum activities were recorded in methanolic extracts. Hydrogen peroxide scavenging activity, reducing power radicals, and DPPH free radical-scavenging activity revealed high activities in P. florida at aqueous and methanol of 500µl/ml extract concentration. The maximum percentage of activity in reducing power assay in H. ulmarius was at 500µl/ml extract and minimum concentration of 100µl H2O2 assay also performed in respective mushrooms. The efficiency of both edible mushrooms poses potential candidature for antioxidant properties in the forthcoming generation.

Keywords: *Pleurotus florida*, *Hypsizygus ulmarius*, Nutrition, Minerals, Vitamins, Antioxidant activity.

INTRODUCTION

Edible mushrooms are valuable healthy foods having rich sources of vitamins B3 and vitamins, are mostly present in both mushrooms. Proteins and minerals especially in potassium and phosphorus. They are also low in calories and fats (Feeney *et al*., 2014; Ozturk *et al*., 2011). *Pleurotus* mushrooms are used in a variety a functional food with a pleasing flavour and scent nutritive and therapeutic properties. As macrofungi, mushrooms usually belong to the Basidiomycotina and occasionally to the Ascomycotina phylum. Basidiomycetes mushroom have been valued as both food and medicine for thousands of years. Many genera of mushrooms are edible and are rich in essential nutrients such as carbohydrates, proteins, vitamins, mineral, fat, fibers, and various amino acids (Okwulehie and Odunze, 2004). A major chunk of the population consumes mushrooms because of their easy availability, flavour, meaty taste, and medicinal value. Mushrooms are large enough to be seen with the naked eye and pulled by hands are considered either hypogeous or epigeous. Edible mushrooms that are grown all over the world (Knop *et al.*, 2015). Although many diseases including cancer, rheumatoid arthritis, cirrhosis, and arteriosclerosis as well as age-related degenerative processes become brought on by the uncontrolled release of oxygen-derived free radicals. Mushrooms have become well-known globally for their nutritional and therapeutic properties (Aditya *et al*., 2024).

However, antioxidant supplement or antioxidant containing foods may be used to help the human body to prevent oxidative damage (Halliwell and Gutteridge 1984; Gulcin *et al.*, 2002; Mau *et al.*, 2001). In most countries, there is a well-established consumer acceptance for cultivated mushrooms (*Agaricus bisporus*, *Pleurotus* sp., *Lentinus edodes*, *Volvariella volvacea*, *Auricularia* sp.). A variety of wild edible mushrooms are traditionally consumed in various parts of the world (Diez and Alvarez, 2001). The antioxidant property in various species of fruits, vegetables, herbs, cereals sprouts and seeds have been examined over the last few decades (Gulcin *et al.*, 2002, Kahkonen *et al*., 1999). Many edible species are important to business as they have been

used for food and medicine for many years and because they contain a diverse range of macromolecules with biological and nutritional qualities that play important functions in biology, which are thought to be the main regulators of antioxidant activities. The edible mushroom is also responsible for antioxidant capacity and biological activities. Eukaryotic, non-photosynthetic organisms having distinct fruiting bodies are mushrooms. They have been used by human beings as food and medicine for thousands of years (Wasser, 2002). The widespread use of mushrooms can be attributed to their wonderful flavour and aroma as well as their significant vitamin and protein content and the molecules they contain that the antioxidants and natural free of pesticides products (Royse 2005). Radicals can donate electrons to other molecules or accept electrons, thus demonstrating oxidation/reduction properties. The most prevalent free radicals associated with various diseases include hydroxyl radical, DPPH radical, superoxide anion radical, hydrogen peroxide, nitrite radical, and peroxy-nitrite radical (Mwangi *et al.*, 2022).

Natural antioxidants are actively studied for their capacity to guard cells and organisms from damage carried on by oxidative stress that the believed to be a contributing factor of degenerative diseases. The moderate, rainy climate of north Turkey's Mid Black Sea region is perfect for mushroom growth in the spring and autumn. The wild edible mushrooms that grow in this part of Turkey (Tokat) are widely considered as a delicacy with local. In Turkey, many studies of trace element content of mushrooms have been performed (Demirba, 2000; Sivrikaya *et al*., 2002) but no information is available about their antioxidant properties and there is no information about *in vitro* antioxidant activity of different extract of mushroom. As a result, worldwide mushroom cultivation has grown significantly during the past few decades (Prabu and Kumutha kalavalli, 2014). To evaluate the antioxidant properties of methanol extracts of some wild edible mushrooms (*Pleurotus florida* and *Hypsizygus ulmarius*) including hydrogen peroxide scavenging (H_2O_2), DPPH and Reducing power assay were determined with standard ascorbic acid and IC_{50} values were calculated.

MATERIALS AND METHODS

Sample collection site

In the present study, fresh healthy spawn of *Pleurotus florida* and *Hypsizygus ulmarius* strain were procured from M.M. Mushroom Industry, Karanthai, Thanjavur, Tamil Nadu, India.

Nutritional content

1. Proximate analysis

Proximate composition analysis of mushrooms was carried out to estimate the percentage of ash, moisture, carbohydrates, protein, fat, and fiber as described by Association of Official Analytical Chemists (AOAC 2006).

2. Vitamin analysis

The vitamins of B (B1, B2, B3, B5) and vitamin C were analysed following procedures by Association of Official Analytical chemists (AOAC 2006).

3. Mineral analysis

The mineral content of calcium and magnesium contents were determined by EDTA versanate complexometric titration method as described by (Harbone 1973). Potassium and sodium ion contents were determined by flame photometry as described by Onwuka (Onwuka, 2005). Zinc, copper, iron, and phosphorous were determined using atomic absorption spectrophotometer (Amadi, 2013).

Preparation of the Mushroom Extract

For the determination of antioxidant activity, *P. florida* and *H. ulmarius* were cultivated on paddy straw substrate and harvested. The freshly obtained fruiting bodies were shade dried and ground into a fine powder. Mushroom extraction was carried out using aqueous and methanol solvents and used for the estimation of antioxidant activities (Trease and Evans, 1983)

4. Antioxidant activity

Reducing power assay: The reducing power assay was determined by the Oyaizu method. Various concentrations of each extract: 100, 200, 300, 400 and 500 µl/ml (2.5 mL), phosphate buffer (2.5 mL, 0.2 M, pH 6.6), and 1% potassium ferricyanide (2.5 mL) were mixed and incubated at 50°C for 20 min. 10% TCA (2.5 mL) was added to the mixture. The mixture was centrifuged at 3,750×g for 10 min. The supernatant (2.5 mL) was mixed with 2.5 mL of deionized water and 0.5 mL of 0.1% ferric chloride.

After 10 min of incubation, the absorbance was measured at 700 nm against a blank (Oyaizu 1996).

5. DPPH Radical-Scavenging Activity: The DPPH radical scavenging activity was estimated. Briefly, a 2.0 ml of aliquot of test sample was added with 2.0ml of 0.16 ml DPPH solution. The mixture was vortexed for 1 min and left to stand at room temperature for 30 min in the dark and the absorbance was recorded at 517nm. Synthetic antioxidant, ascorbic acid, was used as positive controls (Yen and Chen 1995). The ability to scavenge the DPPH radical was calculated using the formula,

Radical scavenging effect (%) = Ab-As / Ab× 100

Where, Ab = Absorbance of blank, As = Absorbance of sample.

6. Hydrogen Peroxide Scavenging: Solution of 0.2 M potassium dihydrogen phosphate and 0.2 M sodium hydroxide solutions were prepared as per the Indian Pharmacopoeia, 1996 standards. Potassium dihydrogen phosphate solution 50 ml was placed in a 200 ml volumetric flask and 39.1 ml of 0.2 M sodium hydroxide solution was added and finally volume was made up to 200 ml with distilled water and phosphate buffer (pH-7.4). 50 ml of phosphate buffer solution was added to equal amount of hydrogen peroxide and generated the free radicals and solution was kept aside at room temperature for 5min of complete the reaction. Extracts (1 ml) in distilled water were added to 0.6 ml hydrogen peroxide solution and the absorbance was measured at 405 nm in a spectrophotometer against a blank solution containing phosphate buffer solution without hydrogen peroxide. The percentage of scavenging of H_2O_2 of mushroom extract was measured. The ability of scavenge the H_2O_2 radical was calculated using the following equation (Agrawal *et al*., 2021).

H_2O_2 scavenging activity (%) = $(A_0 – A_1) / A_0 \times 100$

Where,

A_0 absorbance of the control and A_1 absorbance in the presence of extract sample. A standard ascorbic acid was also same concentrations as that of extract.

RESULT AND DISCUSSIONS

Nutritional value

The proximate composition of the edible mushroom of *Pleurotus* sp. varied. The nutritional content of these mushrooms depends on the species and source as well as their capacity to absorb nutrients from the substrates. In the dehydrated fruiting bodies, carbohydrates constituted the largest portion followed protein, ash, fiber, and fat content. *Pleurotus* sp. exhibited the highest carbohydrate content and energy value. The significant ash content in *Pleurotus* sp. may enhance its abundance of minerals and vitamins. The highest fiber percentage, complementing the dietary fiber intake, whereas *P. florida* boasts the highest carbohydrate and energy level, measuring 320 Kcal per 100 g of dry weight. Many factors influence the nutritional make up of edible mushrooms including the substrate composition, which is also highlighted (Belewu, 2003).

In the present study stated that the proximate composition of *P. florida* and *H. ulmarius*, dehydrated fruiting bodies, and moisture constituted the largest portion with ash, carbohydrates, Protein, fat, and fiber followed by *P. florida* exhibited the highest protein content and energy value. The energy contribution of *P. florida* and *H. ulmarius* was higher because of the higher content of moisture. The high ash content of *P. florida* may contribute to its rich minerals and vitamins content. *P. florida* highest percentage of fiber supplementing the fiber intake in the diet. Overall, these results indicated that the mushrooms are good sources of protein, carbohydrate, minerals, and vitamins, through different nutritional composition richness varies by species.

The approximate composition of edible mushrooms is recorded in **Table 1**. On a dry weight basis, the protein concentration ranged from (13.07%) and *H. ulmarius* (11.01%) was proximate to our result mainly *P. florida*. Slight variation in outcomes might be caused by environmental conditions, nutrition supply, and climate change. The content of highest fat ranged from 6.28%) and lowest fat content in (5.32%) *H. ulmarius* respectively. At *P. florida* ash content in the range of (23.01%) and lowest value exhibited in *H. ulmarius* (21.05%) of ash content respectively. The highest fiber, carbohydrates and moisture content (9.19%, 11.05%, 69.12%) was analyzed in *P. florida* at lowest fiber, carbohydrates

and moisture content are shown by *H. ulmarius* (8.07%, 18.03%, 65.10%,). These results might vary depending on the environmental conditions, substrate used, mushroom species and drying techniques. Values are expressed as the mean ± SD of triplicates were maintained respectively.

Table 1: Composition of nutritional components in proximate, vitamins and minerals of *P. florida* and *H. ulmarius*

Nutritional components		*P. florida*	*H. ulmarius*
Proximate content	Ash(µg/g)	23.01	21.05
	Moisture (%)	69.12	65.10
	Carbohydrates (µg/g)	11.05	18.03
	Protein (µg/g)	13.07	11.01
	Fat (µg/g)	6.28	5.32
	Fiber (µg/g)	9.19	8.07
Vitamin content (µg/g)	vitamin B	3.05	2.31
	vitamin Bl	5.18	3.12
	vitamin B2	1.17	4.09
	vitamin B3	6.37	5.94
	vitamin B5	2.83	2.38
	vitamin C	6.05	4.97
Mineral content (µg/g)	Calcium (Ca)	2.13	15.3
	Copper (Cu)	1.20	2.25
	Iron (Fe)	3.47	2.21
	Magnesium (Mg)	1.44	4.12
	Phosphorus (P)	2.73	3.43
	Potassium (K)	4.45	1.21
	Sodium (Na)	1.88	2.16
	Zinc (Zn)	1.73	2.21

Vitamin content

The vitamin content of food plays a significant role in determining its overall nutritional value. Mushrooms, abundant in vitamin B but lack the fat-soluble vitamins (A, D, E, and K) (Olasupo *et al*., 2019). It is important to note that the specific vitamin contents vary depending on the mushroom species. In the course of this investigation, we are pleased to present report on the vitamin composition of two species of wild mushrooms that are fit for consumption. The nutritional analysis encompasses the levels of vitamin D, vitamin B1, B2, niacin, folic acid, and vitamin C found within these three species of wild mushrooms. Vitamin C was identified as the most prevalent vitamin among those assessed with niacin, vitamin D, and vitamin B2 following suit. However, vitamin B1 and vitamin B, were found to be present below the detectable threshold may have significant implications for the anti-obesity properties of *Pleurotus* sp in humans (Igile *et al.*, 2020). All two mushrooms have the same levels of vitamin D and vitamin C, with only trace amounts of vitamin B2.

Vitamin C acts as a natural antioxidant, while vitamin D and vitamin B, B3, are crucial for the effective distribution and absorption of nutrients in our body. However *Pleurotus* sp, stands out for its significantly higher niacin content compared to the other two edible mushrooms.

The present investigation suggested that first ever report on the vitamin content of the two edible wild mushrooms *P. florida* and *H. ulmarius* important factor in nutritional value of food and also vitamin content. The vitamin B, B1, B2, B3, B5 and vitamin C content were reported in edible mushrooms **(Table 1)**. Among the evaluated vitamins B, vitamin C was the most abundant vitamin detected followed by vitamin B3, vitamin B5 and vitamin B2, while vitamin B1 and vitamin B are found below the detectable level. The two mushrooms contain minimum amounts of vitamin B and vitamin C, while vitamin B2 is present in trace amount and also vitamin B, vitamin B3, are essential for the proper distribution and absorption of other nutrients inside our body. *P. florida* contains substantially higher niacin (6.37%) in comparison to the other edible mushrooms.

Mineral content

This high phosphorus content in *Pleurotus florida* makes it a valuable contribution to human nutritions (Salami *et al*., 2017).The mineral composition of mushrooms is largely influenced by the accumulation of minerals through the growing mycelium from the substrate undergo compositional modifications (Oliveira 2000) with K, Na, and P being the most commonly found minerals followed by Ca and Fe in smaller amounts to analyze the mineral content in the dried samples of wild edible species *Pleurotus* sp. the proximate mineral levels were determined. Minerals play a crucial role as micronutrients in maintaining the body's proper functioning. Among the species evaluated, *P. florida* exhibited the highest concentration of essential minerals such as calcium (Ca), copper (Cu), potassium (K), magnesium (Mg), sodium (Na), phosphorus (P) and zinc (Zn). In comparison, the mushroom *P. florida* displayed an elevated concentration of iron (Fe) at 79.74ppm, surpassing both *Pleurotus* sp. Amongst the minerals analyzed, K was found to be the most abundant, followed by Fe, Na, Mg, Zn, Ca, Cu and Mn, while P was present in a minimal concentration in all *Pleurotus* sp. Most of the major minerals required for the body are present in all edible mushrooms.

In current study, edible mushrooms are important sources of minerals such as calcium (Ca), copper (Cu), iron (Fe), potassium (K), magnesium (Mg), phosphorus (P), sodium (Na), zinc (Zn) and are summarized in **(Table 1)**. The mineral composition of mushrooms is reported to be largely influenced by the minerals through growing mycelium from the substrate. In most of the cases Cu and Na were reported to be present in significant quantities followed by Cu and P in low quantities. The results of the present study, the proximate mineral content in the dried samples of wild edible species of *P. florida* and *H. ulmarius.* Minerals are micronutrients that are essential for the proper functioning of the body. Amongst the evaluated species *P. florida* possesses the highest concentration of most of the essential minerals (Zn, K, Na, Ca, Mg). K is present in elevated concentration in the mushroom *P. florida* as compared to *H. ulmarius.* K was found to be the most abundant followed by Zn, Cu, Na, Fe, Ca, Mg, while P was present in a minimal concentration of two mushrooms. Most of the major minerals required for the body are present in two wild edible mushrooms.

Antioxidant activity

In previous study all samples reduction power increased based on the concentration dependent and the reducing power of *Pleurotus ostreatus.* Aqueous extracts often showed this higher reducing power than methanolic extracts and extracts from dried samples was more frequent than extracts from fresh samples. The aqueous extract produced by boiling the fruiting body indicated the highest value of reducing power in the case of extracts from dried samples. The fruiting body's aqueous extract of the fruit bodies was produced by boiling it showed the maximum reducing power. Aqueous extract samples have been found to usually exhibited more DPPH radical scavenging activity than methanol extract samples, both in dry and fresh conditions (Ivette Gonzalez-Palma, *et al*., 2016). Edible mushrooms, including wild-harvested or cultivated fungal species, possess excellent organoleptic properties and high nutritional values (Liuzzi *et al*., 2023). *Pleurotus* sp could be attributable to variations in cultivation and storage

conditions, the age at which the samples were collected, as well as to variation in the methods of detection.

The antioxidant properties of reducing power assay in *Pleurotus ostreatus* and using aqueous and methanol solvents. The five concentrations are used in the property such as 100µl to 500µl respectively. The highest values for all antioxidant characteristics, DPPH, reducing power and hydrogen peroxide (H_20_2). The lowest values in antioxidant properties of *P. ostreatus* in 100 µl concentration of aqueous and methanol solvents respectively. The acetone, methanol and hot water extracts of *P. florida* also showed an increase in concentration-dependent reduction power. The acetone extract had the greatest reducing power inhibition, whereas the hot water extract had the lowest. The acetone, methanol and hot water extracts from *P. florida* fruiting bodies have been showed to possess concentration-dependent DPPH radical scavenging properties (Kyung Hoan Im *et al.,* 2014). The methanolic extracts of *Pleurotus* sp. have outstanding reducing properties that continuously increased with concentration. A putative scavenger is incubated with H_2O_2 in an experiment for H_2O_2 scavenging activity and the amount of H_2O_2 lost during the reaction is then measured. The concentration-dependent scavenging activity of the farmed oyster mushroom methanolic extracts in the DPPH assay increased (Dandamudi Rajesh Babu *et al*., 2014). Reducing power by ferricyanide/Prussian blue assay the reducing power method reflects the electron donation ability of antioxidants present in the extracts to convert Fe3+ into Fe2+. The amount of the Fe2+ complex was followed by measuring the formation of Perls' Prussian blue at the absorbance of 690 nm. *A. mellea* from showed a stronger reducing power (IC50 = 0.52 mg/mL), while the lowest one was observed in *M. procera* from (IC50 = 1.11 mg/mL). The results from our *M. procera* were higher than previously reported studies by (Barros *et al*., 2007 and Fernandes *et al*., 2013).

In the current study that the antioxidant properties of hydrogen peroxide (H_20_2), DPPH and reducing power assay, in *Pleurotus florida* and using aqueous and methanol solvents.500 µl concentration of two solvents obtained the highest values for all antioxidant characteristics, and the solvent of aqueous in DPPH assay, then methanol inreducing power assay and lowest values in antioxidant properties of *P. florida* in 100µl concentration of aqueous and methanol also H_2O_2assay, respectively. Similarly, *Pleurotus florida,* has the highest level of these properties present in reducing power assay at aqueous solvent **(Figure 1 and 2)**.

In the present study that the antioxidant properties of hydrogen peroxide (H_20_2) DPPH and reducing power assay, was using aqueous and methanolic solvents. *H. ulmarius* 500µl concentration of two solvents obtained the highest values for all antioxidant characteristics, aqueous and methanol solvent in reducing power assay and lowest value in antioxidant properties of *Hypsizygus ulmarius* in 100 µl concentration of aqueous and methanol solvent in same H_2O_2 assay, respectively. Similarly *Hypsizygus ulmarius* has the highest level of these properties present in reducing power assay at methanol solvent **(Figure 3 and 4)**.

In *pleurotus florida* maximum amount of ascorbic acid present in methanolic solvent at 500µl and minimum amount of aqueous solvent in 100µl. In *Hypsizygus ulmarius* maximum amount of ascorbic acid present in methanolic solvent at 500µl and minimum amount of aqueous solvent value 100 µl concentration **(Figure 2 and 4)**.

In the current investigation for IC_{50} values are the antioxidant activity of highest amount of IC_{50} value in methanol solvent such as *Pleurotus florida* of antioxidant capacity found in Hydrogen peroxide scavenging activity radicals, (IC_{50} 6.09±0.25 %) Reducing power radicals (IC_{50} 2.15±0.52 %) and DPPH free radical-scavenging activity radicals ((IC_{50} 2.96±0.18 %) and another extract of *Hypsizygus ulmarius* in antioxidant capacity. Hydrogen peroxide scavenging activity radicals (IC_{50}4.28±0.22 %)**,** Reducing power radicals (IC_{50} 2.70±0.61 %) and DPPH free radical-scavenging activity radicals (IC_{50} 3.30±0.22%) recorded respectively **(Figure 1 and 3)**. Similarly, *Pleurotus florida and Hypsizygus ulmarius* both are same methanol solvent were obtained the highest antioxidant products in reduction power assay, (24.1±0.42%). (11.3±5.59%).The lowest values in antioxidant properties of *Pleurotus florida* and *Hypsizygus ulmarius* in 100 µl concentration of aqueous solvents respectively. The *pleurotus florida* has the majority of all antioxidant qualities and also highest levels of these properties when compared to *Hypsizygus ulmarius* respectively.

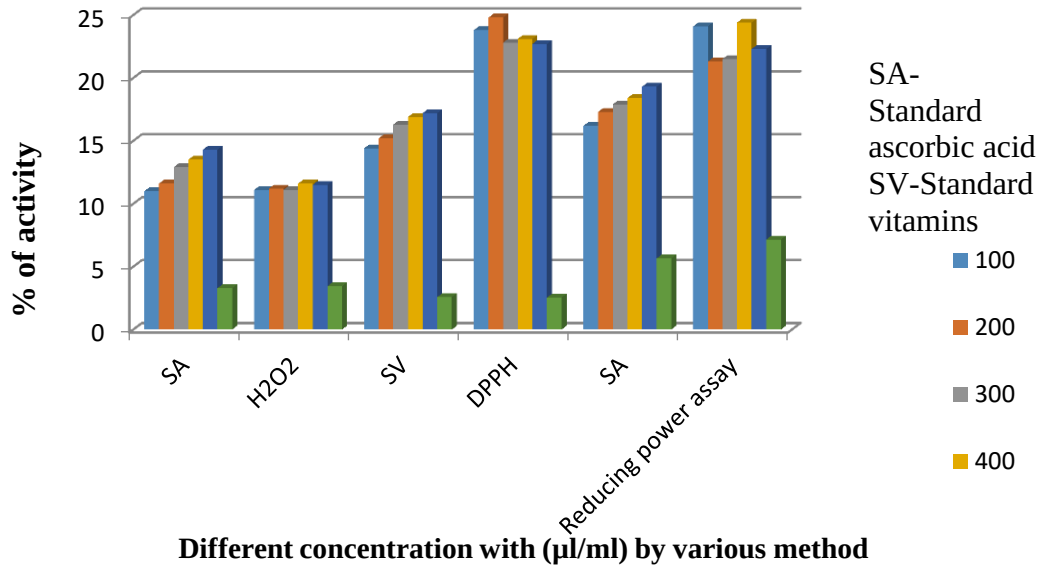

Figure 1: Effect of anti-oxidant activity of *Pleurotus florida* with aqueous extract by different method

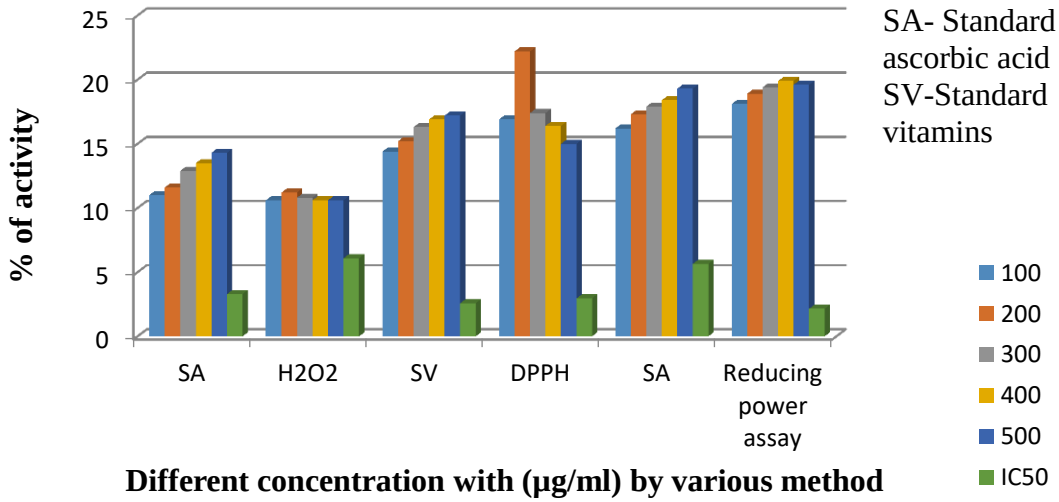

Figure 2: Effect of anti-oxidant activity of *Pleurotus florida* with methanol extract by different methods

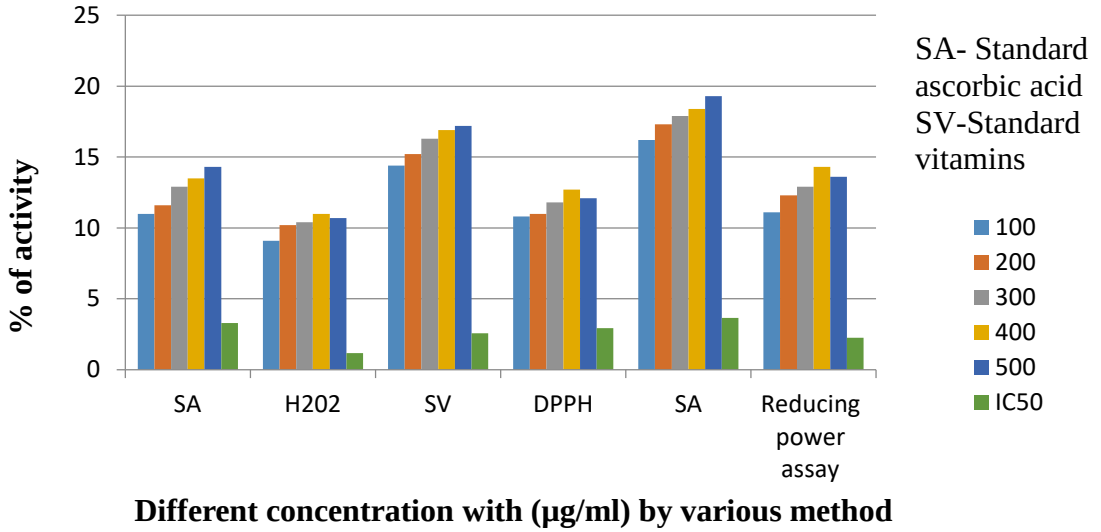

Figure 3: Effect of anti-oxidant activity of *Hypsizygus ulmarius with* aqueous extract by different methods

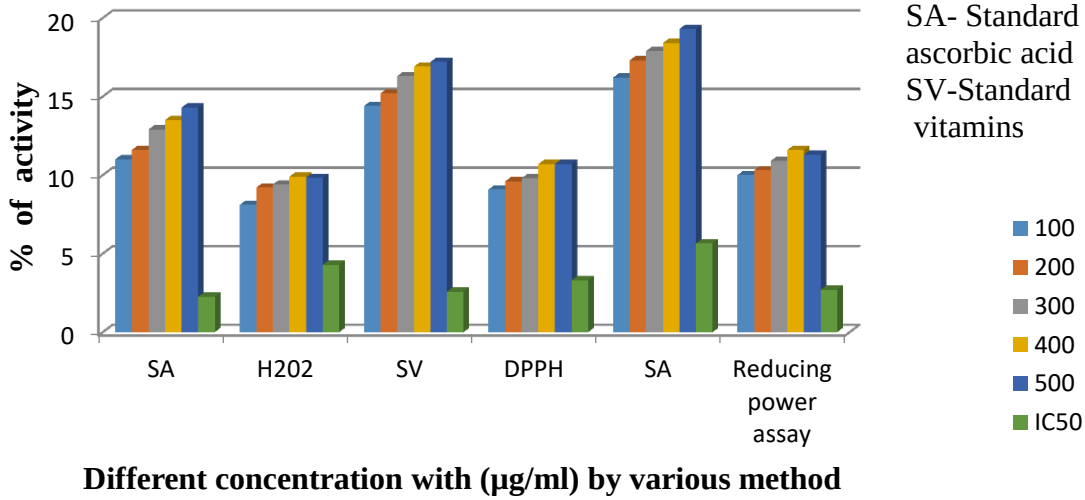

Figure 4: Effect of anti-oxidant activity of *Hypsizygus ulmarius* with methanol extract by different methods

CONCLUSION

In the present investigation, the nutritional and bioactive composition of two edible mushrooms, *Pleurotus florida* and *Hypsizygus ulmarius*, were analyzed. The analyses include the estimation of protein, carbohydrate, fats, ash, minerals, and vitamins, as well as their antioxidant potential. The experimental results revealed that the two wild edible mushrooms are an excellent source of ash, protein, fiber, vitamins, and other essential minerals. In terms of nutritional content, the two mushrooms contain an almost equivalent percentage. Vitamins and minerals in the human diet holds immense significance. *P. florida* and *H. ulmarius* exhibited the high total phenolic and flavonoid content, which corresponds to

their high radical scavenging properties. Overall, the findings from this comparative study provide strong evidence that the chemical composition, nutritional and antioxidant properties of mushrooms can be influenced by the geographic, microclimatic, and edaphic conditions of the collection site. This research significantly contributes to the development of nutritional and pharmaceutical databases for mushrooms that are consumed globally.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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